



# National Institute of Standards & Technology

## Certificate of Analysis

### Standard Reference Material<sup>®</sup> 2392-I

#### Mitochondrial DNA Sequencing (Human HL-60 DNA)

This Standard Reference Material (SRM) is intended to provide quality control when performing the polymerase chain reaction (PCR) and sequencing of human mitochondrial DNA (mtDNA) for forensic identification, medical diagnosis, or mutation detection. It may also serve as a control when amplifying (PCR) and sequencing any DNA. This SRM can also be used for quality assurance when assigning values to in-house control materials. It is certified for the sequences of the entire human mtDNA (16 569 base pairs) from a promyelocytic cell line (HL-60) prepared from the peripheral blood leukocytes from an individual with acute promyelocytic leukemia. A unit of SRM 2392-I consists of 65  $\mu$ L of extracted DNA from cell culture line HL-60 at a nominal concentration of 1.4 ng/ $\mu$ L, which is contained in a vial packaged in a protective plastic box.

**Certified Values:** The certified sequence information of extracted human DNA from HL-60 is provided in Table 1. Also provided in Table 1 is the certified sequence information for two additional entire mtDNA templates, CHR and GM09947A, which are provided in SRM 2392. SRM 2392-I only contains the HL-60 template. Table 2 contains the sequences of 58 unique primer sets that were designed to amplify any portion or the entire human mtDNA [1].

**Supplemental Information:** The sequence information of an additional two DNA templates, (GM03798 [1] and GM10742A [2]), that were amplified and sequenced in their entirety multiple times at NIST are provided in references 1 or 2. Although the extracted DNA from GM03798 and GM10742A are not provided, the cell cultures can be obtained from NIGMS Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research, Camden, NJ. A schematic of the differences from the Cambridge Reference Sequence [3] found in the mtDNA from all five templates is shown in Figure 1.

**Expiration of Certification:** The certification of this SRM is valid until **31 March 2008**, provided the SRM is handled and stored in accordance with the instructions given in this certificate. This certification is nullified if the SRM is damaged, contaminated, or modified.

**Maintenance of SRM Certification:** NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

The analytical determination, technical measurements and analysis of data for the certification of this SRM were performed by D.K. Hancock, K.L. Richie, K.A. Holland (on sabbatical from Gettysburg College, Gettysburg, PA), and B.C. Levin of the NIST DNA Technologies Group, Biotechnology Division.

The overall direction and coordination of the technical measurements leading to the certification was performed by B.C. Levin of the NIST DNA Technologies Group, Biotechnology Division.

The support aspects involved in the issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by B.S. MacDonald of the NIST Measurement Services Division.

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Support for the preparation and certification of this SRM was provided by the National Institute of Justice through the NIST Office of Law Enforcement Standards.

## NOTICE AND WARNINGS TO USER

**Warning:** SRM 2392-I IS A HUMAN SOURCE MATERIAL. SINCE THERE IS NO CONSENSUS ON THE INFECTIOUS STATUS OF EXTRACTED DNA, HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE.

**Permissions:** The research to use HL-60 DNA in SRM 2392-I was deemed exempt from the policy of Part 27 of Title 15 of the Code of Federal Regulations by the NIST Institutional Review Board and the Director of the Chemical Science and Technology Laboratory. This work fit into the exemption category described in 15 CFR 27.101(b)(4) which states: “Research, involving the collection or study of existing data, documents, pathological specimens, or diagnostic specimens, if, these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects.”

ATCC also waived condition 3(c) in their Material Transfer Agreement which states that the “purchaser shall not sell, lend, distribute or otherwise transfer the material or replicates to any others” for the use of HL-60 in the NIST mitochondrial DNA SRM. They stated that, in their view, “as a government agency, NIST will not be providing this material as a commercial product despite the collection of fees for the SRM.”

**Storage:** Store frozen at a temperature of  $-20^{\circ}\text{C}$ . **DO NOT** use a self-defrosting freezer because of periodic cycling of temperatures may shorten the shelf life of this SRM.

## INSTRUCTIONS FOR USE

It is recommended that once thawed, each SRM component should be used in its entirety. Repeated freezing and thawing is **NOT** recommended as this might shorten the shelf life of the SRM. If it is necessary to perform repeated analyses, thaw the SRM and divide the tube contents into aliquots that will be kept frozen until use. Thawing can be conducted at refrigerator temperatures, room temperature, or at  $37^{\circ}\text{C}$ . Once thawed, the sample should be processed without delay. DNA concentrations given are nominal values and are **NOT** intended for use as concentration standards.

## SOURCE AND ANALYSIS<sup>1</sup>

**Source of Material:** DNA from HL-60 was prepared by the Professional Services Department of the American Type Culture Collection (ATCC), Manassas, VA. This material was subsequently vialled at NIST into 65  $\mu\text{L}$  portions (nominal DNA concentration of  $1.4\text{ ng}/\mu\text{L}$ ) and labeled SRM 2392-I Component D (Components A, B, and C are available in SRM 2392).

**NIST Analysis:** PCR was used to amplify the HL-60 DNA in its entirety multiple times using all 58 primer sets. The PCR products were sequenced with an Applied Biosystems, Inc. 310 automated sequencer. The sequences of representative PCR products of the final HL-60 DNA included in SRM 2392-I were reanalyzed to ensure sequence accuracy.

**Interlaboratory Analyses:** An interlaboratory evaluation of the amplification, sequencing and data analysis of the HL-60 template was conducted by four laboratories, including NIST. These laboratories were: The Armed Forces DNA Identification Laboratory (AFDIL), Rockville, MD; Federal Bureau of Investigation Laboratory (FBI), Quantico, VA; and The Georgia Bureau of Investigation (GBI), Decatur, GA. The sequences obtained by all of the laboratories were identical. Description of the interlaboratory analysis of HL-60 is described in reference 2.

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<sup>1</sup>Certain commercial equipment, instruments, or materials are identified in this certificate in order to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Table 1. Certified Human mtDNA Sequence Differences from the Cambridge Reference Sequence (CRS) [3,4] Found in the Two Templates (CHR and GM09947A) in NIST SRM 2392 and One Template (HL-60) in NIST SRM 2392-I

| Comparison with the Cambridge Reference Sequence (CRS) |                                 |                              |                                |                                |                      |          |
|--|---------------------------------|------------------------------|--------------------------------|--------------------------------|----------------------|----------|
| CRS  |                                 |                              |                                |                                |                      |          |
| # <sup>a</sup>   | Base <sup>b</sup> 1981/<br>1999 | Template<br>CHR <sup>d</sup> | Template<br>9947A <sup>d</sup> | Template<br>HL-60 <sup>e</sup> | Amino acid<br>change | Region   |
| 73   | A                               | G                            | -                              | G                              |                      | HV2      |
| 93   | A                               | -                            | G                              | -                              |                      | HV2      |
| 150  | C                               | -                            | -                              | T                              |                      | HV2      |
| 152  | T                               | -                            | -                              | C                              |                      | HV2      |
| 195  | T                               | C                            | C                              | -                              |                      | HV2      |
| 204  | T                               | C                            | -                              | -                              |                      | HV2      |
| 207  | G                               | A                            | -                              | -                              |                      | HV2      |
| 214  | A                               | -                            | G                              | -                              |                      | HV2      |
| 263*R  | A                               | G                            | G                              | G                              |                      | HV2      |
| 295  | C                               | -                            | -                              | T                              |                      | HV2      |
| 303-309  | -                               | C (ins)                      | CC (ins)                       | -                              |                      | HV2      |
| 311-315*R  | -                               | C (ins)                      | C (ins)                        | C (ins)                        |                      | HV2      |
| 489  | T                               | -                            | -                              | C                              |                      | HV2      |
| 709  | G                               | A                            | -                              | -                              |                      | 12sRNA   |
| 750 *R   | A                               | G                            | G                              | G                              |                      | 12sRNA   |
| 1438*R   | A                               | G                            | G                              | G                              |                      | 12sRNA   |
| 1719   | G                               | A                            | -                              | -                              |                      | 16sRNA   |
| 2706   | A                               | G                            | -                              | G                              |                      | 16sRNA   |
| 3106-3107*E  | CC/del                          | del C                        | del C                          | del C                          |                      | 16sRNA   |
| 3423*E   | G/T                             | T                            | T                              | T                              | Silent               | ND1      |
| 4135   | T                               | -                            | C                              | -                              | Tyr → His            | ND1      |
| 4216   | T                               | -                            | -                              | C                              | Tyr → His            | ND1 LHON |
| 4769*R   | A                               | G                            | G                              | G                              | Silent               | ND2      |
| 4985*E   | G/A                             | A                            | A                              | A                              | Silent               | ND2      |
| 5186   | A                               | G                            | -                              | -                              | Silent               | ND2      |
| 5228   | C                               | -                            | -                              | G                              | Silent               | ND2      |
| 5633   | C                               | -                            | -                              | T                              |                      | tRNA Ala |
| 6221   | T                               | C                            | -                              | -                              | Silent               | COI      |
| 6371   | C                               | T                            | -                              | -                              | Silent               | COI      |

| Comparison with the Cambridge Reference Sequence (CRS) |                                 |                              |                                |                                |                        |            |
|--|---------------------------------|------------------------------|--------------------------------|--------------------------------|------------------------|------------|
| CRS  |                                 |                              |                                |                                |                        |            |
| # <sup>a</sup>   | Base <sup>b</sup> 1981/<br>1999 | Template<br>CHR <sup>d</sup> | Template<br>9947A <sup>d</sup> | Template<br>HL-60 <sup>c</sup> | Amino acid<br>change   | Region     |
| 6791   | A                               | G                            | -                              | -                              | Silent                 | COI        |
| 6849 <sup>h</sup>                                      | A                               | G(0.3A) <sup>h</sup>         | -                              | -                              | Thr → Ala <sup>h</sup> | COI        |
| 7028   | C                               | T                            | -                              | T                              | Silent                 | COI        |
| 7476   | C                               | -                            | -                              | T                              |                        | tRNA Ser   |
| 7645   | T                               | -                            | C                              | -                              | Silent                 | COII       |
| 7861   | T                               | -                            | C                              | -                              | Silent                 | COII       |
| 8448   | T                               | -                            | C                              | -                              | Met → Thr              | ATPase 8   |
| 8503   | T                               | C                            | -                              | -                              | Silent                 | ATPase 8   |
| 8860*R   | A                               | G                            | G                              | G                              | Thr → Ala              | ATPase 6   |
| 9315   | T                               | -                            | C                              | -                              | Phe → Leu              | COIII      |
| 9559*E   | G/C                             | C                            | C                              | C                              | Arg → Pro              | COIII      |
| 10172  | G                               | -                            | -                              | A                              | Silent                 | ND3        |
| 10398  | A                               | -                            | -                              | G                              | Thr → Ala              | ND3        |
| 11251  | A                               | -                            | -                              | G                              | Silent                 | ND4        |
| 11335*E  | T/C                             | C                            | C                              | C                              | Silent                 | ND4        |
| 11719  | G                               | A                            | -                              | A                              | Silent                 | ND4        |
| 11878  | T                               | C                            | -                              | -                              | Silent                 | ND4        |
| 12071 <sup>het</sup>                                   | T                               | -                            | -                              | C/T <sup>het</sup>             | Phe→Leu <sup>het</sup> | ND4        |
| 12612  | A                               | G                            | -                              | G                              | Silent                 | ND5        |
| 12705  | C                               | T                            | -                              | -                              | Silent                 | ND5        |
| 13572  | T                               | -                            | C                              | -                              | Silent                 | ND5        |
| 13702*E  | G/C                             | C                            | C                              | C                              | Gly → Arg              | ND5        |
| 13708  | G                               | A                            | -                              | A                              | Ala → Thr              | ND5 LHON   |
| 13759  | G                               | -                            | A                              | -                              | Ala → Thr              | ND5        |
| 13966  | A                               | G                            | -                              | -                              | Thr → Ala              | ND5        |
| 14199*E  | G/T                             | T                            | T                              | T                              | Pro → Thr              | ND6        |
| 14272*E  | G/C                             | C                            | C                              | C                              | Phe → Leu              | ND6        |
| 14365*E  | G/C                             | C                            | C                              | C                              | Silent                 | ND6        |
| 14368*E  | G/C                             | C                            | C                              | C                              | Phe → Leu              | ND6        |
| 14470  | T                               | C                            | -                              | -                              | Silent                 | ND6        |
| 14569  | G                               | -                            | -                              | A                              | Silent                 | ND6        |
| 14766*E  | T/C                             | T                            | C                              | T                              | Ile → Thr              | ND6        |
| 15257  | G                               | -                            | -                              | A                              | Asp→Asn                | CYT B LHON |

| Comparison with the Cambridge Reference Sequence (CRS) |                                 |                              |                                |                                |                      |            |
|--|---------------------------------|------------------------------|--------------------------------|--------------------------------|----------------------|------------|
| CRS  |                                 |                              |                                |                                |                      |            |
| # <sup>a</sup>   | Base <sup>b</sup> 1981/<br>1999 | Template<br>CHR <sup>d</sup> | Template<br>9947A <sup>d</sup> | Template<br>HL-60 <sup>e</sup> | Amino acid<br>change | Region     |
| 15326*R  | A                               | G                            | G                              | G                              | Thr → Ala            | CYT B      |
| 15452  | C                               | -                            | -                              | A                              | Leu → Ile            | CYT B      |
| 15812  | G                               | -                            | -                              | A                              | Val → Met            | CYT B LHON |
| 16069  | C                               | -                            | -                              | T                              |                      | HV1        |
| 16183  | A                               | C                            | -                              | -                              |                      | HV1        |
| 16184-93   | -                               | C (ins)                      | -                              | -                              |                      | HV1        |
| 16189  | T                               | C                            | -                              | -                              |                      | HV1        |
| 16193  | C                               | -                            | -                              | T                              |                      | HV1        |
| 16223  | C                               | T                            | -                              | -                              |                      | HV1        |
| 16278  | C                               | T                            | -                              | T                              |                      | HV1        |
| 16311  | T                               | -                            | C                              | -                              |                      | HV1        |
| 16362  | T                               | -                            | -                              | C                              |                      | HV1        |
| 16519  | T                               | C                            | C                              | -                              |                      | HV1        |

<sup>a</sup> Numbers correspond to Cambridge Reference Sequence [3]

<sup>b</sup> Base found in 1981[3] Base found in 1999 [4]

<sup>d</sup> SRM 2392, reference 1

<sup>e</sup> SRM 2392-I, reference 2

- Base pair same as in 1981 Cambridge Reference Sequence [3]

<sup>h</sup> Possible heteroplasmic site. This heteroplasmy seen in the mtDNA from the first CHR cell culture line is not seen in the mtDNA from the second CHR cell culture line. The second CHR cell culture line agrees with the CRS at np 6849. It is DNA from the second CHR cell culture line that is supplied in NIST SRM 2392.

\*R Rare polymorphisms in Cambridge Reference Sequence discovered by reanalysis of original placenta by Andrews et al., 1999 [4].

\*E Error in Cambridge Reference Sequence discovered by reanalysis of original placenta by Andrews et al., 1999 [4].

del Deletion

ins Insertion

het Heteroplasmy found in HL-60 at np 12071

HV1 Non-coding region found from 16024 and 16569

HV2 Non-coding region found from 1 and 576

CHR DNA Sequence based on two amplifications and cycle sequencing procedures with DNA from the first cell culture line and at least one amplification and cycle sequencing procedure with DNA from the second cell culture line.

GM09947A DNA Sequence based on two amplifications and cycle sequencing procedures.

HL-60 DNA Sequence based on two amplifications and cycle sequencing procedures in both the forward and reverse directions for a total of 4 sequences.

ATPase 6 ATP synthase 6

ATPase 8 ATP synthase 8

CYTB Cytochrome B

COI Cytochrome C Oxidase I

COII Cytochrome C Oxidase II

COIII Cytochrome C Oxidase III

LHON Leber Hereditary Optic Neuropathy

ND1 NADH dehydrogenase 1

ND2 NADH dehydrogenase 2

ND3 NADH dehydrogenase 3

ND4 NADH dehydrogenase 4

ND5 NADH dehydrogenase 5

ND6 NADH dehydrogenase 6

Table 2. Reference Sequences for Primer Sets Used for PCR Amplification of Human mtDNA

| Primer Set Number | Primer Sequence |
|-------------------|-----------------|
| 1(HV2)            | F15<br>R484     |
| 2                 | F361<br>R921    |
| 3                 | F756<br>R1425   |
| 4                 | F873<br>R1425   |
| 5                 | F1234<br>R1769  |
| 6                 | F1587<br>R2216  |
| 7                 | F1657<br>R2216  |
| 8                 | F1993<br>R2216  |
| 9                 | F2105<br>R2660  |
| 10                | F2417<br>R3006  |
| 11                | F2834<br>R3557  |
| 12                | F2972<br>R3557  |
| 13                | F3234<br>R3557  |
| 14                | F3441<br>R3940  |
| 15                | F3635<br>R4162  |
| 16                | F3931<br>R4728  |
| 17                | F4183<br>R4728  |
| 18                | F4392<br>R4983  |
| 19                | F4447<br>R4982  |
| 20                | F4797<br>R5553  |
| 21                | F4976<br>R5553  |
| 22                | F5318<br>R5882  |
| 23                | F5700<br>R6262  |
| 24                | F5999<br>R6526  |
| 25                | F6242<br>R6526  |
| 26                | F6426<br>R7030  |
| 27                | F6744<br>R7255  |

| Primer Set Number |        | Primer Sequence                      |
|-------------------|--------|--------------------------------------|
| 28                | F7075  | GAGGCTTCATTCACTGATTTC                |
|                   | R7792  | GGGCAGGATAGTTCAGACGG                 |
| 29                | F7215  | CGACGTTACTCGGACTACCC                 |
|                   | R7792  | GGGCAGGATAGTTCAGACGG                 |
| 30                | F7645  | TATCACCTTTCATGATCACGC                |
|                   | R8215  | GACGATGGGCATGAAACTG                  |
| 31                | F7901  | TGAACCTACGAGTACACCGACTAC             |
|                   | R8311  | AAGTTAGCTTTACAGTGGGCTCTAG            |
| 32                | F8164  | CGGTCAATGCTCTGAAATCTGTG              |
|                   | R8669  | CATTGTTGGGTGGTGATTAGTCG              |
| 33                | F8539  | CTGTTCGCTTCATTATTGCC                 |
|                   | R9059  | GTGGCGCTTCCAATTAGGTG                 |
| 34                | F8903  | CCCCTTCTTACCACAAGGC                  |
|                   | R9403  | GTGCTTTCTCGTGTTACATCG                |
| 35                | F9309  | TTTCACTTCCACTCCATAACGC               |
|                   | R9848  | GAAAGTTGAGCCAATAATGACG               |
| 36                | F9449  | CGGGATAATCCTATTTATTACCTCAG           |
|                   | R9995  | AGAGTAAGACCCTCATCAATAGATGG           |
| 37                | F9754  | AGTCTCCCTTACCATTTCGG                 |
|                   | R10275 | AAAGGAGGGCAATTTCTAGATC               |
| 38                | F10127 | ACTACCACAACCTCAACGGCTAC              |
|                   | R10556 | GGAGGATATGAGGTGTGAGCG                |
| 39                | F10386 | GGATTAGACTGAACCGAATTGG               |
|                   | R11166 | CATCGGGTGATGATAGCCAAG                |
| 40                | F10704 | GTCTCAATCTCCAACACATATGG              |
|                   | R11267 | TGTTGTGAGTGTAATTAGTGCG               |
| 41                | F11001 | AACGCCACTTATCCAGTGAACC               |
|                   | R11600 | CTGTTTGTCTGATAGGCAGATGG              |
| 42                | F11403 | GACTCCCTAAAGCCCATGTCTG               |
|                   | R11927 | TTGATCAGGAGAACGTGGTTAC               |
| 43                | F11760 | ACGAACGCACTCACAGTCG                  |
|                   | R12189 | AAGCCTCTGTTGTCAGATTAC                |
| 44                | F11901 | TGCTAGTAACCACGTTCTGGTG               |
|                   | R12876 | GATATCGCCGATACGGTTG                  |
| 45                | F12357 | AACCACCCTAACCCTGACTTCC               |
|                   | R12876 | GATATCGCCGATACGGTTG                  |
| 46                | F12601 | TTCATCCCTGTAGCATTGTTCTG              |
|                   | R13123 | AGCGGATGAGTAAGAAGATTCC               |
| 47                | F12793 | TTGCTCATCAGTTGATGATACG               |
|                   | R13343 | TTGAAGAAGGCGTGGGTACAG                |
| 48                | F13188 | CACTCTGTTCGCAGCAGTATG                |
|                   | R13611 | TCGAGTGCTATAGGCGCTTGTC               |
| 49                | F13518 | CATCATCGAAACCGCAAAC                  |
|                   | R13935 | TGTGATGCTAGGGTAGAATCCG               |
| 50                | F13715 | GAAGCCTATTTCGAGGATTTT                |
|                   | R14118 | TGGGAAGAAGAAAGAGAGGAAG               |
| 51                | F13899 | TTTCTCCAACATACTCGGATTC               |
|                   | R14388 | TTAGCGATGGAGGTAGGATTGG (New Primer)  |
| 52                | F14189 | TTAGCGATGGAGGTAGGATTTCG (Old Primer) |
|                   | R14189 | ACAAACAATGGTCAACCAGTAAC              |
| 53                | R14926 | TGAGGCGTCTGGTGAGTAGTGC               |
|                   | F14470 | TCCAAAGACAACCATCATTCC                |
| 54                | R14996 | CGTGAAGGTAGCGGATGATTTC               |
|                   | F14909 | TACTCACCAGACGCCTCAACCG               |
| 55                | R15396 | TTATCGGAATGGGAGGTGATTTC              |
|                   | F15260 | AGTCCCACCCTCACACGATTTC               |
|                   | R15774 | ACTGGTTGTCTCCGATTTCAGG               |

| Primer Set Number |        | Primer Sequence        |
|-------------------|--------|------------------------|
| 56                | F15574 | CGCCTACACAATTCTCCGATC  |
|                   | R16084 | CGGTTGTTGATGGGTGAGTC   |
| 57 (HV1)          | F15971 | TTAACTCCACCATTAGCACC   |
|                   | R16451 | GCGAGGAGAGTAGCACTCTTG  |
| 58                | F16097 | TACATTACTGCCAGCCACCATG |
|                   | R336   | TTAAGTGCTGTGGCCAGAAG   |
| -21M13            | F      | TGTAACACGACGGCCAGT     |

HV2: Hypervariable region 2

HV1: Hypervariable region 1

F: forward primer

R: reverse primer

These are the same primers used for SRM 2392 and reference 1 except the reverse primer of set 51 has been changed to: TTAGCGATGGAGGTAGGATT**GG**. The change (C to G) occurs at np 14368 and is in bold and underlined. Those using SRM 2392 should also use the new reverse primer 51.

#### REFERENCES

- [1] Levin, B.C.; Cheng, H.; Reeder, D.J.; *A Human Mitochondrial DNA Standard Reference Material for Quality Control in Forensic Identification, Medical Diagnosis, and Mutation Detection*; Genomics, Vol. 55, pp. 135-146 (1999).
- [2] Levin, B.C.; Holland, K.A.; Hancock, D.K.; Coble, M.; Parsons, T.J.; Kienker, L.J.; Williams, D.W.; Jones, MP.; Richie, K.L.; *Comparison of the Complete mtDNA Genome Sequences of Human Cell Lines - HL-60 and GM10742A - from Individuals with Promyelocytic Leukemia and Leber Hereditary Optic Neuropathy, Respectively, and the Inclusion of HL-60 in the NIST Human Mitochondrial DNA Standard Reference Material - SRM 2392-I*; Mitochondrion, Vol. 2, pp. 386-399 (2003).
- [3] Anderson, S.; Bankier, A.T.; Barrell, B.G.; deBruijn, M.H.L.; Coulson, A.R.; Drouin, J.; Eperon, I.C.; Nierlich, D.P.; Roe, B.A.; Sanger, F.; Schreier, P.H.; Smith, A.J.H.; Staden, R.; Young, I.G.; *Sequence and Organization of the Human Mitochondrial Genome*; Nature, Vol. 290, pp. 457-465 (1981).
- [4] Andrews, R.M.; Kubacka, I.; Chinnery, P.F.; Lightowlers, R.N.; Turnbull, D.M.; Howell, N.; *Reanalysis and Revision of the Cambridge Reference Sequence for Human Mitochondrial DNA*; Nature Genetics, Vol. 23, p. 147 (1999).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or via the Internet <http://www.nist.gov/srm>.



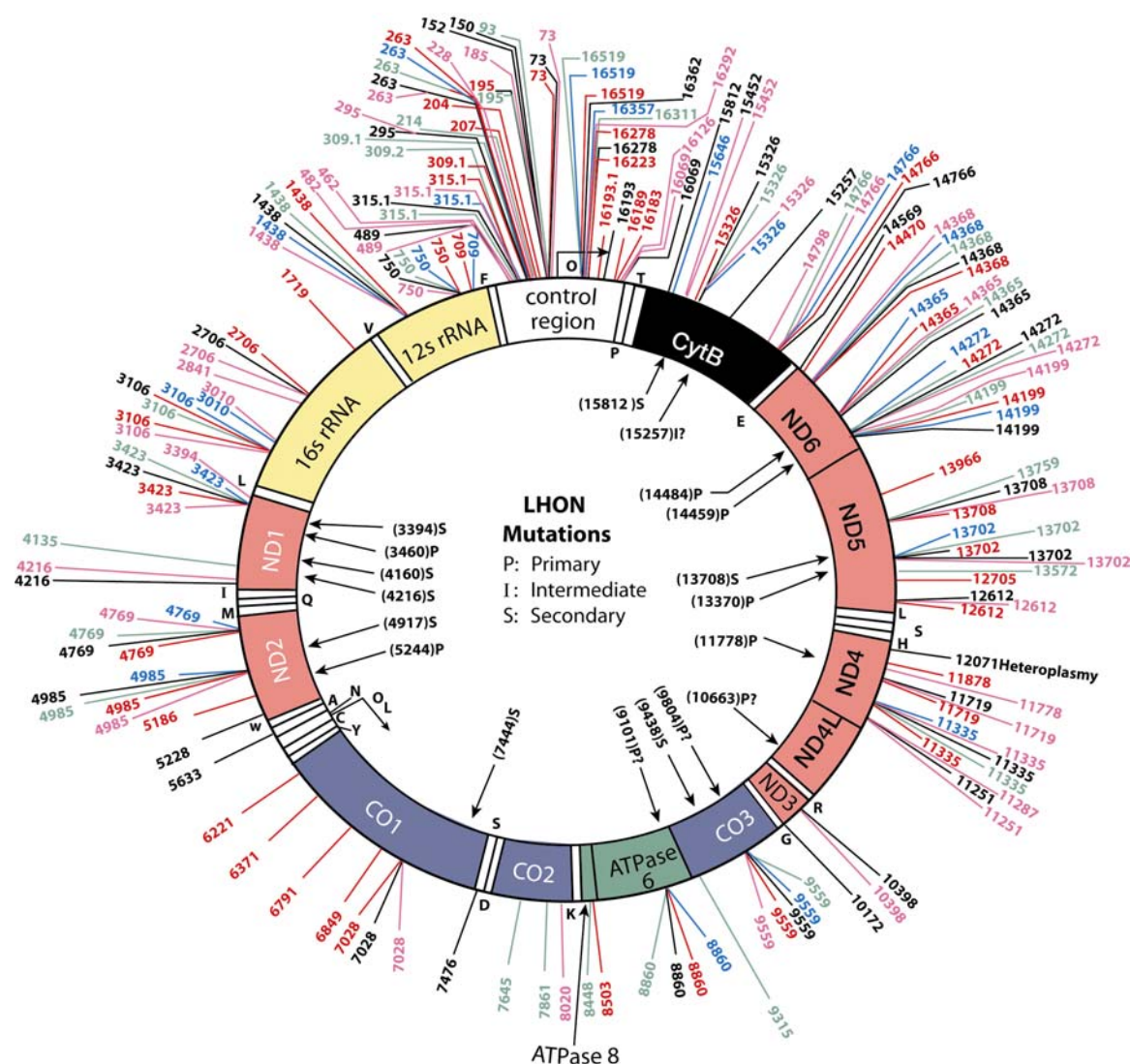


Figure 1. Schematic of human mtDNA showing its circular double-stranded DNA and all the differences from Cambridge Reference Sequence (1981) found in CHR (red), 9947A (green), HL-60 (black), GM03798 (blue), and GM10742A (purple) as numbers along the outside of the color-coded circle. Locations of the control region, rRNAs and genes coded by human mtDNA are shown. The locations of the 22 tRNAs are noted by white areas in the circle and designated by their single letter amino acid code. Since a number of mutations found in GM10742A and HL-60 and one change in CHR have been associated with primary, intermediate or secondary mutations linked to the disease Leber Hereditary Optic Neuropathy (LHON), the position of these mutations plus other LHON mutations are shown on the inside of the circle (Wallace et al., 1997). The question mark following the np of the LHON mutations indicates the assignment is not confirmed. One of the primary mutations that have been associated with LHON, G11778A, was found in GM10742A [2] but not found in the other DNA templates examined in this research. (Modified from Levin et al., 1999).